A comparison of recirculation aquaculture systems versus biofloc for on-growing of juveniles of *Tinca tinca* (Cyprinidae) and *Mugil cephalus* (Mugilidae).

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Abstract

The on-growing of tench *Tinca tinca* (1.81 ± 0.6 g) and grey mullet *Mugil cephalus* (0.65 ± 0.2 g) fry was carried out using two different culture systems, recirculation (RAS) and biofloc (BFT), to compare their performance and evaluate the feasibility of rearing both species using an alternative method. After an on-growing period of 50 days, it was possible to verify that the survival rate, fish size in terms of body weight and length, condition factor (K), specific growth rate, final biomass and apparent feed conversion rate of *M. cephalus* fry were significantly higher ($P < 0.05$) in RAS in comparison to those obtained using BFT. For *Tinca tinca*, results were similar for all the measured variables except for the K, that was significantly higher in BFT ($P < 0.05$). Water quality parameters remained within the optimum ranges reported for both freshwater fish species using RAS. In BFT, despite the constant addition of glucose, total ammonium concentrations were relatively high (2.89 ± 1.25 mg/L for tench and 3.74 ± 1.34 mg/L for mullet) because of the small volume of water in tanks (90 L) and the use of an inert diet with high protein levels (>50%). Ammonia could only be stabilized when the feed was replaced with one with a lower protein content (35%). The proximate composition of the bioflocs showed that the composition varied according to the fish species considered, with mullet the protein (17.34 ± 1.40%) and fat (2.36 ± 0.03%) were present in higher concentration than in tench (8.92 ± 0.38% and 2.18 ± 0.18%, respectively), indicating that regardless of the use of the same BFT procedures, bioflocs developing for both species were different. The microbial diversity in the tank water and the intestinal microbiota of fish were examined by restriction fragment length polymorphism (RFLP) and found to be different depending on the system used for on-growing. Thus, in the RAS system the microbial diversity was somewhat higher than in the BFT. As a conclusion, present results indicated that *M. cephalus* fry seemed to grow better using RAS, whereas *Tinca tinca* seem to be able to adapt to the BFT systems.

Keywords: RAS; BFT; minimal water exchange; proximate composition; RFLP.

1. Introduction

Among the aquaculture technologies developed to preserve natural resources with minimal water exchange, recirculation aquaculture systems (RAS, Verdegem et al., 2006; Dalsgaard et al., 2015) and biofloc (BFT, De Schryver et al. 2008; Ekasari et al., 2015) are widely used. According to Losordo et al. (1998) and Piedrahita (2003), RAS are cultures of high structural and technological complexity, where water circulates several times a day through biological and ultraviolet filters in order to allow high densities of animals to be propagated with drastically reduced dependence on water supplies. These systems are usually implemented with extensive environmental controls for pH, foam fractionators, solids decanters, carbon dioxide removal and continuous application of oxygen, whereas ammonia nitrogen is controlled by the balance of nitrifying bacteria in the biological filter. On the other hand, according to Avnimelech (2009) and

Crab et al. (2012), in BFT systems a small part of the total water volume circulates through solid decanters and some water is added to compensate for losses by evaporation, while in RAS minor daily water exchanges (<10%) result only for the purpose of system maintenance (*i.e.* filter cleaning by flushing and removing the nitrate produced by nitrification). In addition, the immobilization of water ammonia is carried out through the addition of biodegradable organic carbon, because suitable ratios of the organic carbon to the nitrogen available in the water (C/N) stimulate the growth and proliferation of heterotrophic bacteria (Avnimelech, 1999). An additional benefit of BFT systems in comparison to RAS and open-flow systems is the possibility of taking advantage of the microbial biomass as a food supplement, resulting in the use of feed with lower protein concentration (Hargreaves, 2006). Thus, according to some authors (Azim and Little, 2008; Crab et al. 2012), biofloc technology can become an interesting alternative to recirculation, which requires high protein content feed and complex filtration systems. Biofloc technology have proved suitable to produce tilapia *Oreochromis niloticus* and the shrimp *Litopenaeus vannamei* (Avnimelech, 2007; Azim and Little, 2008; Luo et al., 2014). However, investigations have been made to verify the feasibility of farming species other than those listed under the BFT system: *Macrobrachium rosembergii* (Crab et al., 2010), *Rhamdia quelen* (Poli et al., 2015) and *Carassius auratus* (Wang et al., 2015).

Common tench, *Tinca tinca*, is widely appreciated in European countries for its taste, white meat and polyunsaturated fatty acid content (Vácha and Tvrzická, 1995). It is considered one of the most promising species for the development of freshwater aquaculture (Kujawa et al., 2011). Its culture is relatively easy due to its omnivorous habits and its adaptation to lentic environments of high turbidity and low oxygen levels (Steffens, 1995), characteristics that favor its production in earthen tanks, and probably in BFT systems as suggested by Carbó and Celades (2011). Its attractive market price has stimulated efforts to make large-scale production viable (IPAC, 2006); however, studies related to the intensification of this species are still scarce (Celada et al., 2009, Garcia et al., 2015). On the other hand, grey mullet *Mugil cephalus* is an economically important euryhaline and eurythermal species that has been recognized as a potential species for aquaculture diversification in the Mediterranean region, as well as in other regions of the world (Republic of Korea, Taiwan Province of China, South Africa), because of its good adaptation to captivity, rapid growth, omnivorous feeding habits and high market price of its salt-cured and dried eggs named "bottarga" (Whitfield et al., 2012). Due to its omnivorous and detritivorous feeding habits and its high tolerance to low water quality, it is presumed that this species could be grown in BFT systems.

The objective of the present study was to compare the zootechnical performance of tench and mullet juveniles during on-growing in water recirculation (RAS) and biofloc (BFT) systems.

2. Material and methods

2.1 Animals and diets

On-growing of tench and mullet fry was carried out at IRTA facilities in San Carlos de la Rapita (Tarragona, Spain) during May and June of 2016. Juveniles of both species *T. tinca* (1.81 ± 0.16 g) and *M. cephalus* (0.65 ± 0.2 g) were transported to IRTA by road from the Regional Aquaculture Center Vegas de Guadiana (Extremadura, Spain) and Roset Angulas from the Delta del Ebro (Tarragona, Spain), respectively. Before the experiment, fish were kept during three weeks in open circuit, at ambient temperature and fed *ad libitum* a mixture of commercial feed with variable protein content. They were on-grown under RAS and BFT using 2.2 kg/m^3 initial density. The fresh water used, with an electrical conductivity of 2500 µS/cm, was collected from the subsoil through an artesian well 40 m deep and then treated with sodium hypochlorite. The experimental units consisted of 16 cylindrical fiberglass 90 L tanks; thus, 4 tanks were used for tench in RAS and 4 in BFT (n= 110 fish/tank), and similarly, 4 tanks for RAS and another 4 for BFT were allocated for mullet (n= 306 fish/tank). Fish culture was performed at room temperature (22-25°C). Due to the unavailability of suitable feed for tench, fish were fed "MP-M Pearl"

(Skretting, Spain), with 56% crude protein, 15% crude fat and 1.1 to 1.3 mm diameter (data provided by the feed manufacturer), at a feeding rate of 4.5% of the biomass divided into five servings per day (08, 11, 14, 17 and 20h). In the case of grey mullet, the diet used was "Perle Eel Proactive" (Skretting, Spain), with 54% crude protein, 24% crude fat, 20.7 Mj/kg digestible energy and 0.7 mm diameter (data provided by the feed manufacturer), at 10% of the biomass, also divided into five servings per day. As ammonia levels could not be stabilized in BFT due to the high protein levels of diets, both feeds were replaced at the fourth week of the trial with one feed with a lower protein content (tilapia diet "LE-F TI3", Skretting, Spain; proximate composition: 35% protein, 6% fat, 13 Mj/kg digestible energy). The diet had a diameter of 1.9 mm and was manually crushed and sieved through sieves of 0.5 and 1.0 mm.

2.2 Growing systems

The IRTAmar® RAS systems, recirculation modules capable of recycling up to 0.5 kg of food with a protein level of 50 % and volumes of 4,500 L per hour, were used for the trial. Each module is composed of sand and cartridge filters (5 µm), a biological filter (submerged bed), an ultraviolet filter (60W) and sensors for measuring dissolved oxygen, temperature and water flow levels. The water recirculation rate was set at twice the volume of each unit per hour (180 L/tank/hour), at a renewal rate of 10% of the total volume per day (9 L/tank/day). For the BFT culture system, the biofloc did not come from an initial inoculum, but it was formed *de novo* due to the contribution of food and the absence of water renewal in tanks, which stimulated microbial growth and biofilm formation. The activity of the heterotrophic bacteria was stimulated with the daily addition of anhydrous glucose (organic carbon content = 46%). To calculate the amount of carbohydrate to be added, the criterion recommended by Avnimelech (1999) was followed. In some cases, when total ammonium reached a concentration higher than 6 mg/L, 50% of the volume of water was renewed. Decanters with capacity equal to that of the culture tanks were driven through air-lift when the total solids concentration exceeded 500 mg/L. Due to the natural production of epidermic mucus by tench juveniles, cotton strips (10% of the surface area) were introduced into the on-growing tanks to trap the accumulated mucus and to promote the growth of biofilm (Bratvold and Browdy, 2001, Browdy and Moss, 2005, Schveitzer et al., 2013).

2.3 Water quality parameters

Temperature, dissolved oxygen, pH, total ammonia, nitrite and total suspended solids (TSS) were determined daily, whereas alkalinity, nitrate and volatile suspended solids (VSS) were analyzed once per week (Table 1). Total ammonia, considered a critical parameter in BFT systems, was measured in two ways: once a week by means of the indophenol method (Strickland and Parsons, 1972) using microplates and absorbance read at 240 nm (Infinite M200 spectrophotometer; Tecan Trading AG, Switzerland), and daily with a Merck colorimetric kit (MColortest™). Due to the water turbidity of the biofloc systems, all samples were diluted 1/10 to better visualize the colorimetric card. The values of the colorimetry test were correlated with those of the analytical method to generate an equation to correct the subjectivity of the colorimetric method. The values of non-ionized ammonium (NH_3) were calculated from the concentration of total ammonium (NH_4^+), pH and temperature of water (Boyd, 1990).

2.4 Restriction fragment length polymorphism analysis for microbiota diversity analyses

Samples of five fish of each species (grey mullet and tench) were collected from each of three tanks in each treatment group (3 RAS tanks and 3 BFT tanks; n = 15 per treatment). Intestines were dissected from fish after euthanasia by overdose (400 ppm) of tricaine methanesulfonate (MS-222, Sigma-Aldrich, Alcobendas, Spain). Dissected tissues were immediately fixed in 70% ethanol and stored at 4 °C until analysis. Prior to extraction, tissue samples were washed with buffered peptone water to remove traces of ethanol. Then, the tissue was minced into small pieces using sterile scissors and then placed into a 15 mL tube with a small aliquot of zirconium glass beads (1.0 mm diameter, BioSpec Products). This was shaken by hand vigorously for 3-5 minutes to obtain a uniform homogenate. Approximately 400 µL of this homogenate was starting material for each DNA extraction.

Water samples were also collected from the tanks of each group, and a sample of the biofilm material from the biofloc tanks was also collected. Bacterial sludge from the biofilm was collected into a pellet by centrifugation and the supernatant removed prior to DNA extraction. The water samples were filtered using 0.2 µm membrane filters and the filters were cut into small strips to fit into a microcentrifuge tube for DNA extraction of adherent cells. DNA was extracted from all samples using the DNA Stool Mini Kit (Quiagen) following the manufacturers protocol. Extracted DNA was evaluated for purity by spectrophotometry utilizing the ratio of absorbance at 260/280 nm to confirm absence of residual protein content. A fragment of approximately 600 bp (size varies with taxa) of the 16S rDNA from total bacteria of the gut was amplified in a volume of 50 µL using primers previously described (Gomez-Conde et al., 2007; Lane et al., 1991): 5'-CTACGGGAGGCAGCAGT-3' and 5'-CCGTCWATTCMTTTGAGTTT-3'. Each reaction included 100 ng of the gut DNA and had a final concentration of 2 mM $MgCl_2$, 1mM dNTP´s (0.25 mM each), and 0.2 mM of each primer. Amplification conditions were 94 °C for 4 min followed by 35 cycles of 94 °C for 1 min, 45 °C for 1 min (with an increase of 0.1 °C each cycle), followed by 72 °C for 1 min 15 sec. The program finished with a final extension step of 5 min at 72 °C. After amplification, from this total of 50 µL of PCR solution there were 5 different restriction enzyme digestions performed using Alu I, Hha I, Hpa II, Rsa I, and Sau 3AI (New England Biolabs). Each restriction enzyme digestion contained 6 µL of amplified 16S rDNA and an equal volume of digestion premix containing 5 units of enzyme and 2X reaction buffer. This was mixed and incubated for 2 hours at 37 °C. Reactions were stopped by incubation at 80 °C for 20 minutes. The resulting 12 µLs of restriction digests were run on 2% agarose gels at 65 V/cm for 1h. The final gel image was analyzed using GeneTools (SynGene).

2.5 Proximate composition and lipid class of bioflocs

Concentrated biofloc was freeze dried for 24 h and kept at -20 °C until analysis. For homogenization, samples of the freeze dried bioflocs were diluted in distilled water and homogenized during 5 min with an Ultraturrax T-25 (IKA® WERKE, Germany) and sonicated for 1 min (Vibra-cell, Sonics, USA). Protein and carbohydrate content were estimated in triplicates by colorimetric analysis following the methods by Lowry et al. (1954) and Dubois et al. (1956), respectively. Samples for the protein analysis were previously digested with NaOH (40 mg m/L at 60 °C for 30 min). Total lipids from concentrated biofloc were extracted in chloroform:methanol (2:1, v:v) using the method of Folch et al. (1957), and quantified gravimetrically after evaporation of the solvent under a stream of nitrogen followed by overnight vacuum desiccation. Total lipids were stored in chloroform:methanol (2:1, 20 mg/mL) with 0.01% butylhydroxytoluene (BHT) at –20 °C until final analysis. Lipid class separation was performed by high-performance thin-layer chromatography (HPTLC) following the method by Olsen and Henderson (1989). After separation, bands were identified by charring the plates at 100 °C for 30 min after spraying with 3% (w/v) aqueous cupric acetate containing 8% (v/v) phosphoric acid and quantified by scanning densitometry using a GS 800 Calibrated Densitometer (Bio-Rad, Bio-Rad Laboratories, Inc, Hercules, CA, USA).

2.6 Zootechnical parameters

Once a week, *ca.* ~20% of the original population for each species were measured for monitoring growth in body weight (BW) and to recalculate the amount of food to be offered. At the end of the experiment, the same number of individuals of each species and each experimental unit was weighed (Sartorius BP211D, Spain) and their standard length (SL) determined to the nearest 0.1 g and 1 mm, respectively. The specific growth rate (SGR) was calculated with the equation SGR (%/day) = [(lnBWf - lnBWi) x 100] / time (days), where BWf was the final body weight and BWi the initial body weight (g). The condition factor (K) was determined as K = (BWf x 100)/SL^3), where BWf was the final body weight (g) and SL the standard length (cm). Survival rate was calculated by multiplying the difference between the final population and the initial population by 100. All accidental mortalities (diseases in the case of mullet and branchial obstruction with epidermic mucus in the case of tench) were not considered for the calculation of the final survival. Apparent feed conversion rate (A-FCR) was obtained by dividing the total of the feed distributed into tanks between the final biomass reached in each experimental unit.

2.7 Statistical analyses

Results were expressed as mean ± standard deviation (SD) (*n* = 3). Survival, final body weight, standard length, SGR, condition factor and apparent FCR values of each species, as well as water quality parameters of each culture system, were statistically analyzed using a one-way ANOVA and a post-hoc Tukey test, at a significance level of 0.05. All data were checked for normality (Kolmogorov–Smirnov test) and homogeneity of variance (Bartlett's test). The arcsine square root transformation was conducted on data expressed as a percentage. The relationship between water ammonium levels measured by the colorimetric kit and the analytical method were analyzed by means of linear regression. The software Statistica 13 (Dell Statistica Inc., USA) was used for all the analyses.

3. Results and discussion

All recorded water quality parameters, except for dissolved oxygen, total ammonium (NH_4^+) and SST, had stable values for each of the species considered and culture systems studied (Table 2), and remained within the ranges considered suitable for most freshwater species (Boyd, 1990; Timmons et al., 2009).

The levels of oxygen dissolved in the BFT tanks of *M. cephalus* were significantly higher ($P < 0.05$) than those registered in the RAS due to the incorporation of pure oxygen into experimental tanks to cover the high oxygen demand caused by the bacterial respiration of the bioflocs, especially during the application of glucose (Avnimelech, 1999). However, only in the BFT tanks of mullet pure oxygen addition was required, probably due to the increased demand of oxygen caused by the swimming activity of grey mullet juveniles, significantly higher than the tench, whereas mullet approached very excited to the surface to receive the food, the tench remained lethargic in the bottom of the tanks.

The correlation between the ammonium concentrations recorded with the colorimetric kit and those of the analytical method showed that the kit overestimated 0.35 ± 0.26 mg NH_4^+/L (0.05 to 1.14 mg/L) and underestimated 0.63 ± 0.31 mg NH_4^+/L (0.04 to 1.15 mg/L). Despite this discrepancy, the use of the kits was very helpful because of the amount of analysis that needed to be performed daily. The ammonium data in Table 2 correspond to the values adjusted by the equation $y = 0.3769x + 0.5487$ ($R^2 = 0.62$), where y is the total corrected ammonia and x the total ammonium recorded by the colorimetric kit. Total ammonium (NH_4^+) concentration was always higher in BFT rearing tanks in both grey mullet and tench, than in RAS cultures. In biofloc-based cultures, ammonia can be controlled by the application of carbohydrates (Avnimelech, 1999); however, due to the small volume of water used and the high protein concentrations of feeds, the glucose requirements were so high that the oxygen concentration was compromised, even dividing the addition of glucose in several doses throughout the day.

The presence of small concentrations of nitrite (0.58 ± 0.71 for tench and 0.49 ± 0.15 for mullet) in the BFT treatments of both species suggests that the developing flocs in rearing tanks were not always dominated by heterotrophic bacteria; nevertheless, the total absence of nitrates calls into question the existence of nitrification (Zhu and Chen, 2001). Very minor changes were observed in the pH (7.6 - 8.3) or alkalinity (198 - 240 mg $CaCO_3$/L) of the water. It is known that the nitrification processes within the biological filters, and the strong metabolic activity of the heterotrophic bacteria of the BFT systems, consume $CaCO_3$ ions (Ebeling et al., 2004); however, the high buffering capacity (alkalinity of 250 mg/L and hardness of 650 mg/L) seems to have been sufficient to maintain the stability of this parameter in both tested fish culture systems.

The resulting dendrograms (Fig. 1) from the RFLP analyses showed somewhat consistent patterns in that clades formed among fish from the same species and in some cases also from the same treatment group. The overall trend was that more bands were observed from those fish grown using RAS (Table 4). The water and biofilm samples of BFT tended to fall as outliers, but water samples formed a clade separate from the BFT biofilm sample using the enzymes Hha I. In some cases, some fish samples grouped together with the water samples (*e.g.* – Alu I). Using the enzyme Hha I, there was a clear grouping of fish by species even though the banding pattern suggested very different composition of the microbiota between treatment groups. Using the enzyme Rsa I, there was accordance between treatment groups for grey mullet and to a lesser degree also tench, but there was one outlier of a tench biofloc sample, which formed a clade together with the tench RAS samples. The clades which formed using RFLP analysis were suggestive of the impact that the two different treatments/culture methods have on the gut microbiota. However, it is not the complete picture as this does not convey quantitatively the differences in microbial diversity that develops within the gut. There was also an increase in the number of bands obtained from samples derived from the RAS culture systems (Table 3). This increase in the number of bands may correlate to a greater diversity of bacteria in the sample. Focusing on the grey mullet grown in RAS, the uniqueness of the diversity can be inferred by observing the discrete clades formed using three of the five enzyme digestions (Alu I, Hpa II, and Rsa I). As mentioned above, more bands were obtained with samples from RAS, which correlates with more diversity evident by PCR. Quorum sensing may play a role in augmenting the growth of some species at the expense of others leading to apparent reduction in biodiversity in BFT. The BFT microbiota may reflect this selection through quorum-based mechanisms (Schryver et al., 2008). Provided the caveat that this increase in diversity does not include opportunistic pathogens, this improvement of microbial diversity may be of benefit to the host fish species. However, if the contrary is true (some of the increased diversity is composed of potential opportunistic pathogens), then the increased microbial diversity may put the fish host at greater risk for intestinal infections, gastroenteritis, and possible septicemia if other stressors are imposed on the fish culture, which exacerbate pathogen infections generally (*i.e.*, reduced water quality, improper diet, overcrowding, handling and transport stress, among others) (Winton, 2016). In this work, the water samples usually partitioned as outgroups in the cladistic analysis, which it is not surprising and has been reported elsewhere (Giastis et al., 2015). Host-specificity for particular microbial species is modulated by selective pressures within the host gut attributed to gut habitat (i.e. physiology, anatomy) and host's genotype (Navarrete et al. 2012). While in water, a lower abundance of the predominant gut taxonomic groups might be expected, as conditions in water are suboptimal for the growth of defecated bacteria mostly due to the ecological preference of the latter for the gut habitat (i.e. pH, anoxic conditions, etc.), adhesion sites and nutrient availability therein (Giatsis et al., 2015). However, further research is needed for evaluating microbiota from these distinct types of culture systems in more detail (i.e. - microbiome sequencing) for evaluating the impact of RAS and BFT systems on the composition of the microbiota.

According to the biofloc biochemical content and lipid class from grey mullet and tench cultures (Tables 4 and 5), regardless of the use of the same feed during part of the trial (tilapia diet "LE-F TI3", 35% protein), protein content was higher in the case of the grey mullet biofloc, probably due to the feed residues present in the water, whereas for tench these residues were much lower. The same can be said regarding lipid content, having in mind that most of the lipids in the biofloc system were triglycerides with a very high amount of free fatty acids that indicated catabolism of these nutrients (Carey et al., 1983). It seems that the biochemical components of the biofloc were mostly due to feed residues (either in the form of protein or fat) present in the water and fecal production by the juveniles, having in mind that in the case of mullet where SST levels were 2 times higher than in tench.

Data about survival, weight, specific growth rate, biomass, standard length, condition factor and apparent food conversion rate are shown in Table 6. At the beginning of the experiment, grey mullet juveniles reared both in RAS and BFT were affected by an outbreak of *Aeromonas salmonicida* that was treated with oxytetracycline for seven days. In the case of the fish cultured in biofloc, the antibiotic was administered in the feed whereas for the fish in RAS, the system was stopped for an hour, and 5 ppm of the antibiotic were added to the water. Before reactivating the system, all the water in the tanks was renewed. The dead specimens were not replaced. Although mortality was observed in both culturing systems, survival in BFT was significantly lower than in RAS, probably due to the higher concentration of ammonium, which may have had an adverse effect on the immune system of the fish (Colt and Armstrong, 1981). In the case of tench, a high (12 and 18%) and sudden mortality was observed in 2 of the BFT tanks at the beginning of the experiment with the fish showing a gasping behavior, despite the high level of dissolved oxygen and relatively low ammonia. Mucus accumulated in the water was presumably responsible for the obstruction of the gills. After a 50% water renewal, mortality ceased. Strips of cotton, covering approx. 10% of the surface, were installed with the aim of filtering and removing mucus naturally produced by this species (Benzer et al., 2010).

The lower growth rate and the higher apparent FCR observed in both species cultured using BFT may be a consequence of the sublethal concentrations of non-ionized ammonium: 0.16 ± 0.06 mg/L in mullet and 0.13 ± 0.05 mg/L in tench. According to Sampaio et al. (2002), juveniles of *M. platanus* diminished their growth when exposed to 0.08 mg/L NH_3 and concentrations of 3.01 and 0.06 mg/L of total ammonium and of non-ionized ammonium, respectively, are considered as safe for this species. No literature is available regarding the effects of non-ionized ammonia on tench. Gomulka et al. (2011) indicate a maximum of 0.59 mg/L NH_3 for juveniles of the *Leuciscus cephalus* cyprinid. Habbas (2006) found an LC_{50} of 1.11 mg/L NH_3 (96h, pH 8.5) in juveniles of *Cyprinus carpio*, which might indicate that a sublethal effect could manifest above 0.11 mg/L (10%). Although the 20:1 C/N ratio (Avnimelech, 1999) was used, the amount of glucose added was not enough to immobilize the ammonium through the production of bacterial biomass. Similar technical constraints were also reported by Azim and Little (2008) in the cultivation of tilapia in BFT systems with 250 L tanks, where the concentrations of this compound reached critical levels despite the constant addition of carbohydrates.

No deformations were observed in tench, a fact that would indicate no overfeeding and that the feed offered, although formulated for another species, was nutritionally appropriate (Rennert et al., 2003; Wolnicki et al., 2006). It is noteworthy that, unlike mullet, tench maintained in biofloc showed a significantly higher condition factor when compared to those of RAS, which seemed to indicate direct utilization of the biofilm that accumulated on the artificial substrates as a nutrient resource (Bratvold and Browdy, 2001, Azim et al., 2002, Azim and Asaeda, 2005), which could also be associated to the grasping behavior of this cyprinid species. It was also observed that the apparent FCR of the tench in BFT was 1.75 times higher than in RAS, whereas in the mullet the difference increased to 2.78 times. Although tench is considered as a slow growing species among other cyprinids, its development may benefit from the presence of natural food, mainly zooplankton (De la Vega et al., 2007). In fact, this type of food was present in the bioflocs of tench in the form of protozoa, rotifers and some nematodes. The larger diameter of the bioflocs (1.0-3.0 mm in tench vs. 0.08-0.35 mm in grey mullet) may also have favored the supply of food resources (De Schryver et al., 2008). In addition, the values of apparent FCR and K obtained in the BFT treatment suggested that *T. tinca* used the microbiota from the biofloc and growing on the artificial substrates as a food source. Except for the presence of substrates for mucus water removal, results from the present study were similar those found by Wang et al. (2015) with *Carassius auratus* cultivated in BFT, where 100% survival rates, SGR of 0.94 to 1.33%/day and K of 2.74 to 3.09% were reported, supporting the idea that cyprinid species are good fish candidates for being grown in BFT systems

As a conclusion, tench juveniles seemed to have a higher potential to be grown in BFT systems. However, for large-scale trials, the natural production of mucus by the fish in a system where there is virtually no water renewal can represent a significant bottleneck. In the case of grey mullet, recirculation systems seemed to be a better option than biofloc for on-growing purposes. However, experiments with a better control of ammonium nitrogen production should be performed during the early developmental stages of this species to consider or disregard this technology.

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Table 1. Water quality parameters, frequency and analytical methods used.

Parameter	**Frequency**	**Method**
Temperature (°C)	Daily	Oximeter DO 450, Eutech Instruments
Dissolved oxygen (mg/L)	Daily	Oximeter DO 450, Eutech Instruments
pH	Daily	Multi 9310, WTW
Ammonia colorimetry (mg/L)	Daily	Nessler, MColortest™, 0.05-0.8 mg/L NH_4^+
Ammonia indophenol (mg/L)	1 x week	Strickland and Parsons (1972)
Nitrite (mg/L)	Daily	Sulfanilamide, MColortest™, 0.025-0.5mg/L NO_2^-
Nitrate (mg/L)	1 x week	JBL Test NO_3, 0.5- 250 mg/L NO_3^-
TSS (mg/L)	Daily	Gravimetry, 100°C (APHA 2005-2540 E)
VSS (mg/L)	1 x week	Gravimetry, 500°C (APHA 2005-2540 E)
Alkalinity (mg/L)	1 x week	Titration (APHA 2005-2320 B)

Table 2. Water quality parameters (mean ± SD) during the ongrowing of *Tinca tinca* and M*ugil cephalus* using BFT and RAS systems.

	Culture system	T (°C)	DO (mg/L)	pH	NH_4^+ (mg/L)	NH_3 (mg/L)	NO_2^- (mg/L)	NO_3^- (mg/L)	TSS (mg/L)	VSS (mg/L)	Alkalinity (mg/L)
Tench	BFT	22.3 ± 0.89	7.99 ± 0.69	7.93 ± 0,35	2.89 ± 1.25[a]	0.13 ± 0.05[a]	0.58 ± 0,71	0.0	199.2 ± 140.9	57.2 ± 71.6	211.8 ± 18.1
	RAS	22.0 ± 1.19	8.71 ± 0.30	7.98 ± 0,10	0.57 ± 0.36[b]	0.02 ± 0.01[b]	0.31 ± 0,36	21.1 ± 4.7	-	-	225.2 ± 11.9
Mullet	BFT	22.8 ± 0.95	9.24 ± 0.95[a]	7.84 ± 0,17	3.74 ± 1.34[a]	0.16 ± 0.06[a]	0.49 ± 0,15	0.0	360.1 ± 248.1	100.7 ± 68.9	212.5 ± 17.7
	RAS	22.0 ± 1.19	7.70 ± 0.67[b]	7.86 ± 0,13	0.74 ± 0.33[b]	0.03 ± 0.01[b]	0.53 ± 0,27	20.3 ± 5.1	-	-	225.9 ± 14.4

Values are means ± SD. Within the same species, significant differences (*P* <0.05) between culture systems are indicated by different superscripts.

Table 3. Summary of the number of bands occurring for each restriction digestion for each sample. RAS samples are indicated with shading.

		Restriction Enzyme				
		Alu I	**Hha I**	**Hpa II**	**Rsa I**	**Sau 3AI**
Mullet	BFT-1	5	9	7	8	12
	BFT-2	5	4	8	5	10
	BFT-3	7	4	9	6	10
	RAS-1	11	9	9	12	9
	RAS-2	11	9	8	12	8
	RAS-3	9	7	8	12	11
Tench	BFT-1	6	9	7	11	6
	BFT-2	6	10	6	10	6
	BFT-3	8	7	6	6	8
	RAS-1	7	6	4	9	11
	RAS-2	7	9	10	11	11
	RAS-3	7	7	8	8	11
	RAS water	1	4	4	2	3
	BIOFILM	3	4	6	6	6
	BFT water	1	2	3	2	2

Table 4. Biochemical composition of biofloc from mullet and tench cultures.

	Water content (%)	Protein (% DW)	Carbohydrates (% DW)	Lipids (% DW)
Mullet				
Sample 1	3.50 ± 0.42	17,95 ± 1.17	7.95 ± 0.03	1.60 ± 0.34
Sample 2	0.24 ± 0.17	17.34 ± 1.40	19.25 ± 0.54	2.36 ± 0.03
Tench	0.51 ± 0.17	8.92 ± 0.38	12.32 ± 0.68	2.18 ± 0.18

Sample 1 was collected 22 day after to begin the experiment. Sample 2 and sample of tench were collected 30 days after starting the experiment.

Table 5. Lipid class of bioflocs (% of total lipids) from mullet and tench cultures

	Mullet		Tench
	Sample 1	Sample 2	
Total PL	1.89 ± 0.37	10.13 ± 0.81	7.57 ± 0.28
CHOL	22.32 ± 1.38	19.47 ± 0.86	20.37 ± 0.54
FFA	49.45 ± 1.78	15.66 ± 1.34	35.12 ± 1.47
TAG	10.14 ± 1.94	36.83 ± 1.70	17.68 ± 0.76
SE+W	16.21 ± 1.39	17.90 ± 1.01	19.27 ± 1.32
Total NL	98.11 ± 0.37	89.87 ± 0.81	92.43 ± 0.28

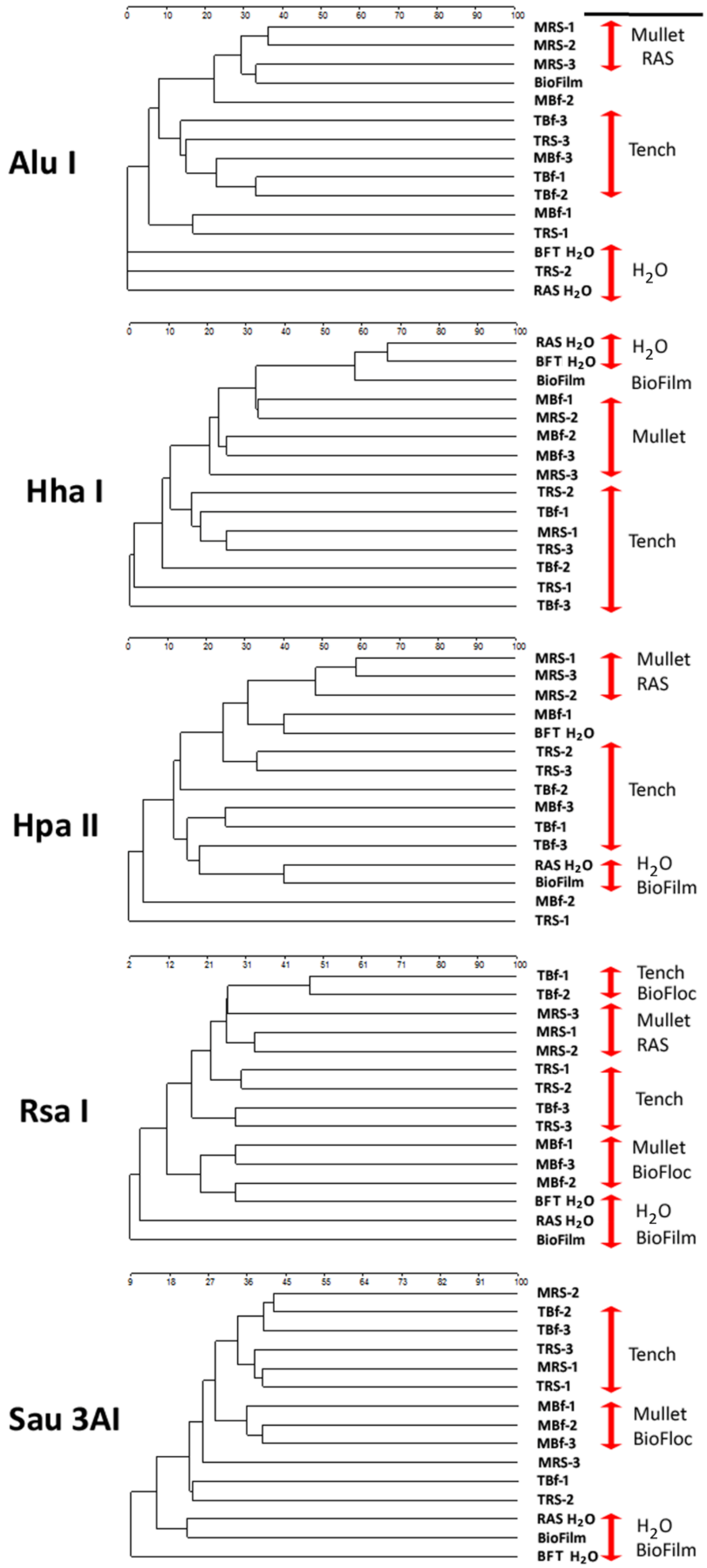
PL: Total phospholipids, CHOL: Cholesterol, FFA: Free fatty acids, TAG: Triacyl glycerol, SE+W: Sterol esters and waxes, NL: Total neutral lipids. Mullet sample 1 was collected 22 day after the beginning of the experiment. Mullet sample 2 and sample of tench were collected 30 days after the beginning of the experiment.

Table 6. Survival (%), body weight (g), SGR (%/day), biomass (kg/m³), standard length (cm), condition factor (K) and apparent FCR of *T. tinca* and *M. cephalus* fry cultured during 50 days in recirculation (RAS) and biofloc (BFT) systems.

	Culture system	Survival (%)	Weigh (g)	SGR (%/day)	Biomass (kg/m³)	Standard length (cm)	K	A-FCR
Tench	BFT	91.6 ± 3.00[b]	3.28 ± 1.60[b]	1.14 ± 0.10[b]	3.68 ± 0.13[b]	5.17 ± 0.74	2.22 ± 0.25[a]	0.42 ± 0.02[b]
	RAS	98.2 ± 1.50[a]	4.14 ± 1.31[a]	1.61 ± 0.04[a]	4.96 ± 0.73[a]	5.65 ± 0.71	2.16 ± 0.15[b]	0.24 ± 0.01[a]
Mullet	BFT	81.1 ± 5.10[b]	1.66 ± 0.76[b]	1.87 ± 0.19[b]	4.57 ± 0.22[b]	3.47 ± 0.60[b]	1.85 ± 0.81[b]	0.53 ± 0.02[b]
	RAS	93.8 ± 0.88[a]	3.40 ± 1.40[a]	3.31 ± 0.20[a]	10.84 ± 0.85[a]	5.48 ± 0.62[a]	2.00 ± 0.12[a]	0.19 ± 0.01[a]

Values are means ± SD. Within the same species, significant differences (P<0.05) between culture systems are indicated by different superscripts.

Figure 1. Dendograms showing clades formed by matching band patterns of each of five restriction enzyme digestions. BFT = biofloc; RAS = recirculation aquaculture system; MRS = mullet from RAS; MBf = mullet from biofloc; TRS = tench from RAS; TBf = tench from biofloc.


Alu I
MRS-1
MRS-2
MRS-3
BioFilm
MBf-2
TBf-3
TRS-3
MBf-3
TBf-1
TBf-2
MBf-1
TRS-1
BFT H_2O
TRS-2
RAS H_2O
Mullet RAS
Tench
H_2O
Hha I
RAS H_2O
BFT H_2O
BioFilm
MBf-1
MRS-2
MBf-2
MBf-3
MRS-3
TRS-2
TBf-1
MRS-1
TRS-3
TBf-2
TRS-1
TBf-3
H_2O
BioFilm
Mullet
Tench
Hpa II
MRS-1
MRS-3
MRS-2
MBf-1
BFT H_2O
TRS-2
TRS-3
TBf-2
MBf-3
TBf-1
TBf-3
RAS H_2O
BioFilm
MBf-2
TRS-1
Mullet RAS
Tench
H_2O BioFilm
Rsa I
TBf-1
TBf-2
MRS-3
MRS-1
MRS-2
TRS-1
TRS-2
TBf-3
TRS-3
MBf-1
MBf-3
MBf-2
BFT H_2O
RAS H_2O
BioFilm
Tench BioFloc
Mullet RAS
Tench
Mullet BioFloc
H_2O BioFilm
Sau 3AI
MRS-2
TBf-2
TBf-3
TRS-3
MRS-1
TRS-1
MBf-1
MBf-2
MBf-3
MRS-3
TBf-1
TRS-2
RAS H_2O
BioFilm
BFT H_2O
Tench
Mullet BioFloc
H_2O BioFilm

Highlights

First report of *Mugil cephalus* and *Tinca tinca* fry biofloc culture.

Mullet fry grow better using RAS system.

Tench fry grow better using BFT system.

Tench maintained in biofloc showed a significantly higher condition factor when compared to those of RAS.